The Cytoplasmic Inclusions of the Germ-Cells.

PART VI. ON THE ORIGIN AND PROBABLE CONSTITUTION OF THE GERM-CELL DETERMINANT OF APANTELES GLOMERATUS, WITH A NOTE ON THE SECONDARY NUCLEI.

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With Plate 9 and 10 Text-figures.

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The posterior pole of the ovum of many Arthropoda, and especially of Holometabolous Hexapoda, has been shown to contain a cytoplasmic aggregation which ultimately becomes

segregated into the germ-cells during early segmentation stages (**4, 15, 21**). On account of this the polar aggregation has been called the germ-cell determinant. In this paper I have made some attempt to ascertain the origin and composition of the germ-cell determinant, and I have also taken the opportunity to draw attention to the remarkable accessory or secondary nuclei.

1. Material and Methods.

The biology of Apanteles and Microgaster has been treated in two other papers (**8, 9**).

Pupæ or newly emerged imagos were found to contain developing eggs in stages old enough for the purpose of this research. The abdomens of the insects were snipped off, teased open and fixed in a variety of fluids; Flemming-without-acetic acid (F.W.A.), Champy, Altmann, Benda, Kopsch (**7**), Golgi, Cajal, Mann, picro-nitric, Carnoy, Gilson-Petrunkewitsch were among the methods tried. Glycogen techniques were also used. The sections were stained in a variety of methods.

2. General Note on the Insect Ovary.

In insects the ovary consists of a number of tubes or ovarioles containing eggs in different stages of development, those nearest the oviduct or the posterior end of the ovariole being ripe, while those at the upper end or apex of the tube are minute. These stages are arranged in a linear series, and between two oöcytes one usually finds a group of nurse-cells (Text-fig. 4). The nurse-cells in most insects appear to be true germ-cells and are often connected to the growing egg by means of cytoplasmic rings, which show that the nurse-cells and the egg they nourish belong to the same cell-generation (**14**). In the ovariole the polarity of the egg is definite; the anterior pole of the egg lies towards the head of the insect, the posterior pole towards the genital opening. The germ-cell determinant is found to appear in the posterior

pole of the egg, while the oöcyte nucleus lies towards the anterior pole of the egg-cell (Text-figs. 1 and 4).

At the apex of the ovariole all the cells are at first similar in appearance and size; as one follows the series down the tube certain cells are found to enlarge, apparently at the expense of the others, and become oöcytes, while the remaining cells either become nurse-cells, or form an epithelium around the oöcyte and are then follicle-cells (Text-fig. 5). According as to whether a germ-cell becomes a follicle, nurse- or egg-cell, the nuclear and cytoplasmic elements become characteristically altered as shall be shown below.

3. Previous Work.

Hegner (**14**) appears to be the only other observer who has examined the oögenesis of Apanteles glomeratus. In his valuable paper Hegner describes how at an early stage the oöcytes acquire an epithelium and are accompanied by a group of nurse-cells. In the nucleus of the young oöcytes the chromatin mass is very large, but later expands to form a spireme, and the nucleus becomes filled with chromatin threads—a condition which persists much later. When the oocytes reach their definitive size the chromatin threads contract into chromosomes, which apparently unite in pairs, as in Copidosoma (also described by Hegner), and become arranged side by side on an asterless spindle. Finally, the chromosomes condense to form a solid lump, as has been shown to occur in many parasitic Hymenoptera (**4**). Some time before the oöcyte nucleus breaks down to form the chromosomes, there appears within the cytoplasm of the anterior half of the oöcyte a great number of spherical bodies which are arranged in and below the zone of the large oöcyte nucleus. These are secondary nuclei, which were first described by Blochmann (**1**) in certain other Hymenoptera, and the substance within them stains like chromatin. The secondary nuclei are present for only a short time, having all disappeared before the nucleus condenses (see **4**, **15**).

In the Diptera, Lepidoptera, Hemiptera and in some Coleoptera, Stuhlmann (**22**) also describes such secondary nuclei.

Blochmann (**1**) considered that the secondary nuclei (he calls them "Nebenkerne") are formed by a process of budding from the oöcyte nucleus. Korschelt (**17**) considered they arose from epithelial cells. Loyez (**18**) thought that the secondary nuclei were not derived from the germinal vesicle by budding, but resulted from a fluid or granulose emission from the oöcyte nucleus. Hegner (**14**) appears to espouse this view, and concludes that "the oöcyte nucleus gives off materials into the cytoplasm which become enclosed by a membrane and develop into nuclear-like bodies." It should also be mentioned that Loyez considers that the secondary

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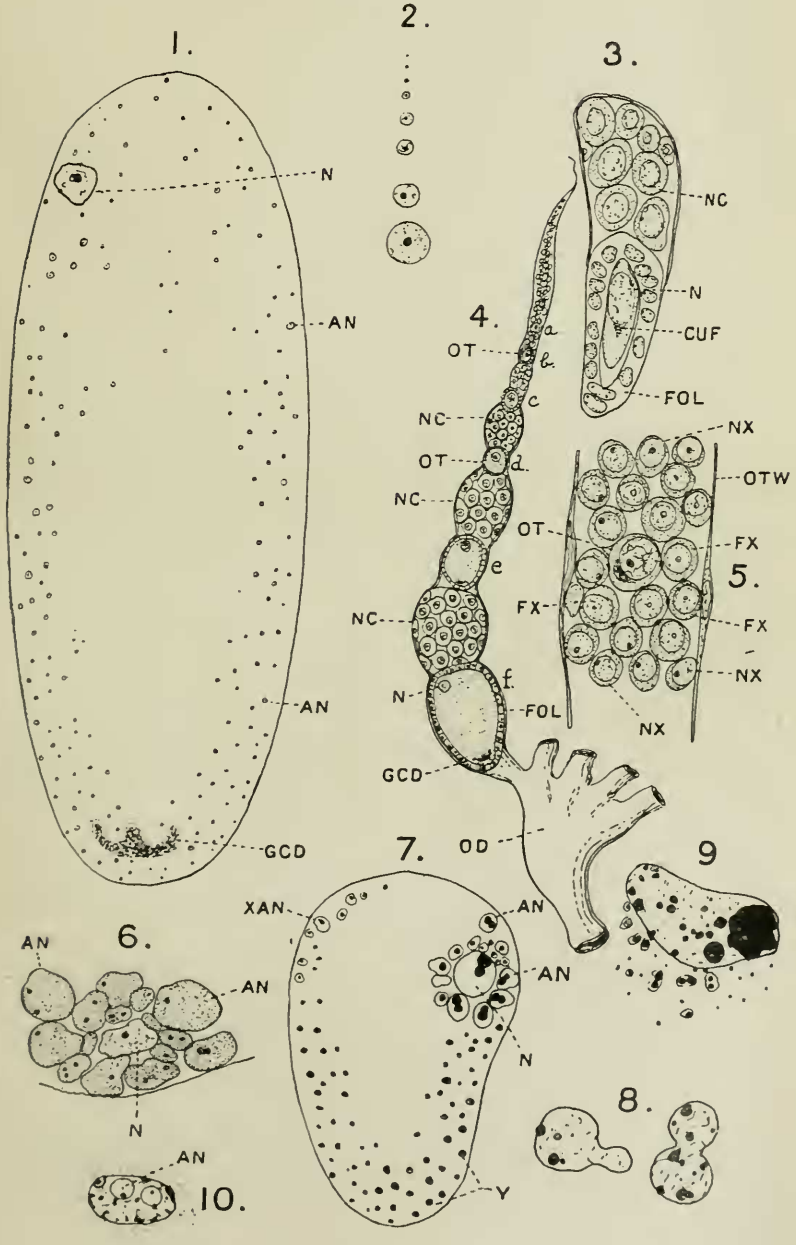
DESCRIPTION OF TEXT-FIGS. 1–10.

Fig. 1.—Diagram of egg of Apanteles glomeratus showing *N.*, the head nucleus; *AN.*, the accessory or secondary nuclei at the periphery of the egg; and *GCD.*, the germ-cell determinant. Fig. 2.—Showing the origin of a secondary nucleus of Apanteles from a solid chromatoid granule. The linin and nuclear membrane develop around the latter. Fig. 3.—Oöcyte and nurse-cells (*NC.*) of Copidosoma Buyssoni (Mayr.), after Silvestri (**21**). At *CUF.* is Silvestri's cloud of granules, supposed to give rise to the germ-cell determinant. *N.* Nucleus; *FOL.* Follicle cells. Fig. 4.—One egg-tube or ovariole of Apanteles glomeratus, showing linear arrangement of developing oöcytes (*OT.*), and groups of nurse cells (*NC.*). At *N.* is the nucleus of the oldest oöcyte, and at *GCD.* the developing germ-cell determinant. *OD.* Oviduct. Fig. 5.—Diagrammatic figure of upper part of ovariole to show origin of oöcyte nurse-cells and follicle-cells. At *OT.* is one inner cell enlarging to form an oöcyte; the cells marked *FX.* will eventually form the cellular layer of the egg (or follicle), while those in the region *NX.* will become nurse-cells. *O.T.W.* Ovarian tube wall. Fig. 6.—Part of oöcyte of Camponotus herculeanus to show primary or head nucleus (*N.*), and around it the secondary nuclei (*AN.*). Note different appearance of latter. After Hegner (**14**). Fig. 7.—Oöcyte of Myrmicina sp. showing the primary nucleus at *N.*, and around it a group of secondary nuclei (*AN.*) These stain like the head nucleus (*N.*) At *XAN.* is a group of secondary nuclei which have wandered from the region of the head nucleus. *Y.* Yolk. After Buchner (**3**). Fig. 8.—Two secondary nuclei of Rhyssa (Ichneumon) to show budding and amitosis. After Buchner (**3**). Fig. 9.—Nucleus of egg of Rhyssa, showing apparent origin of secondary nuclei, by expulsion of nucleolar material from the head nucleus. After Buchner (**3**). Fig. 10.—Nucleus of egg of Rhyssa, showing three accessory nuclei (*AN.*) inside. After Buchner (**3**).

TEXT-FIGS. 1–10.



nuclei may have some function in the formation of the yolk. All observers find that the secondary nuclei sooner or later disappear; exactly how has not been determined.

In other insects Hegner (**15**) has studied the cytoplasmic inclusions—mitochondria and other bodies; this, however, was not done in Apanteles, since his material was fixed in Carnoy and Bouin. In the beetle Leptinotarsa decemlineata, this observer describes the cytoplasm of the very young oöcytes as homogeneous, figuring no inclusions. After a short time the oöcytes form a linear series in the ovariole, and soon there appears in the cytoplasm a number of granules staining with the crystal violet after Benda's method. The cytoplasm growing, becomes filled with more and more granules which become situated near the periphery. The central cytoplasm is of a homogeneous nature, the peripheral crowded with granules and spherical bodies of various sizes. Hegner thinks that the mitochondria lying near the periphery increase in size, swell up, lose their affinity for crystal violet, and finally form the large yolk-granules.

With reference to the mode of origin of the germ-cell determinant in Leptinotarsa, Hegner thinks that the mitochondria might be responsible for its appearance. He could not determine the mode of origin of the germ-disc, but considered that it might be derived from—(1) the cytoplasm of the egg, (2) the cytoplasm of the nurse-cells, (3) the chromatin of the germinal vesicle, (4) chromatin from the nurse-cells, (5) nucleolar substance from the germinal vesicle or nurse-cells or both, and (6) bodies of a mitochondrial nature.

It may be safely remarked that with the exception of yolk and Golgi apparatus, Hegner mentions as the possible source of origin of the germ-cell determinant every likely cell constituent known to cytologists. Hegner apparently does not feel in a position to eliminate from his list any one of the possible sources of origin of the determinant but appears to be attracted by the last section—No. 6.

Silvestri (**21**), using sublimate-alcohol-acetic acid as fixative, describes in Copidosoma buyssoni, a polyembryonic

Chalcid, the origination of the germ-cell determinant as "una sostanza un pò' più colorita dell' ooplasma e meno della cromatina e formante come una sorta di cuffia alla parte posteriore della vesicola. Di questo stadio io ho pochi preparati e non molto belli, perciò non mi credo autorizzato ad affermare in modo assoluto la presenza di tale sorta di cuffia alla parte posteriore della vesicola." In Text-fig. 3 I have copied one of Silvestri's figures, showing at *CUF.* the "sorta di cuffia," which he thinks may have something to do with the origin of the germ-cell determinant. As will be shown below, Silvestri's juxta-nuclear "cuffia" is really unconnected with the germ-cell determinant, but is the partly collapsed remains of the mitochondria. Martin (19) also tries to find some condition inside or near the nucleus to account for the origination of the germ-cell determinant, figuring the germ-cell determinant as appearing on that side of the nucleus which contains a darker mass of chromatoid substance. Martin's evidence is not satisfactory. The chromatoid mass on one side of the nucleus is merely the typical bouquet stage figure of the maturation prophases, and there is no evidence that this is connected with the appearance of the germ-cell determinant.

Paul Buchner (3), in a recent exhaustive study of the accessory nuclei in the hymenopterous oöcyte, has examined Solenius vagus, Andrena sp., Bombus agrorum, Camponotus and Myrmecina sp., Rhyssa and Allantus sp., Arge pagana and Tenthredo mesomelas. In all these forms he finds secondary nuclei. His paper does not enter specially into the questions surrounding the germ-cell determinant. Buchner's conclusions with regard to the secondary nuclei are as follows: "Die akzessorischen Kerne enthalten keine Chromosomen, im übrigen gleichen sie dem Hauptkern, indem sie mit Liningerüst, Nukleolen, Enchylem und Membran versehen sind." Again, "Die akzessorischen Kerne sind auf anfangs nakt im Plasma liegende Chromatingranula zurückzuführen. Um diese entwickelt sich Enchylem Membran und Gerüst, während sie selbst zu den Nukleolen

des akzessorischen Kernes werden." And, "Chromosomen sind auch zur Bildung eines Metazoenkernes nicht unumgänglich nötig, sondern lediglich Chromatin." In addition he considers that "Chromosomen und Nucleolen sind in der Eizelle in hohem Grade unabhängig voneinander."

The last statement is possibly thought to get over a difficulty which I have emphasised below in my discussion on the origin of this chromatin which gives rise to the secondary nuclei (page 148).

Buchner shows clearly that the secondary nuclei originate from grains of chromatin which in some cases are large enough to be found passing through the nuclear membrane. In Text-figs. 7, 8, 9 and 10 I have given four of Buchner's most remarkable figures, illustrating forms which differ from Apanteles, and which give more definite evidence as to the origination of the accessory nuclei.

In another recent paper (**20**), J. Nusbaum-Hilarowicz discusses the behaviour of the mitochondria during the oögenesis of Dytiscus marginalis. He has demonstrated a Golgi apparatus in the egg. In the oögonia there is a cytoplasmic aggregation near the nucleus, which he calls "Idiozomreste," and the mitochondria tend to lie around this region. During the oögonial divisions the mitochondria, which are fine and rod-like, become partly caught up in the astral rays. In Dytiscus the young eggs are connected to the nurse-cells by a zone of cytoplasm, as has been shown by Korschelt (**16**), and Nusbaum-Hilarowicz shows that most of the mitochondria lie in and partially form such cytoplasmic stalks. Besides yolk-granules the author describes the formation of large fat-granules.

The Golgi apparatus was demonstrated by a modification of Kopsch's method as follows:—Fix whole ovary (or ovary + viscera surrounding it) for two hours in this mixture: 1 part of 2 per cent. OsO_4, and 3 parts of corrosive sublimate saturated in normal saline. Then transfer, after slight washing in water, to a 2 per cent. solution of OsO_4. The tube containing the latter is kept ten days in a thermostat at

a constant temperature of + 23° C., afterwards slightly washed in water, dehydrated, cleared, and embedded in wax. Sections may then be treated in turpentine to remove the bulk of the fat stain, if desired.

Except for the raising the temperature this method is similar to one recommended by me elsewhere (7). I find this method does not always succeed, but it does sometimes give results where ordinary Kopsch treatment fails.

4. Secondary Nuclei of Apanteles.

The secondary or accessory nuclei of the oöcyte of Apanteles arise after the egg has grown till it has lost its round shape and become oval and elongate (Text-fig. 4, *e* and *f*). In the most perfect preparations of the ovary that one can make these peculiar bodies are found to arise as minute solid chromatoid granules; they appear in the egg-cytoplasm far removed from the true nucleus. In Text-fig. 2 a number of stages showing the manner of origin of the accessory nucleus are given; at first the chromatic granule is solid and naked, but soon a distinct nuclear membrane appears around it, and the structure looks like a sphere surrounded by a thin ring. Later the extra-spherical ring (nuclear membrane) grows and gradually a linin network may be seen. Eventually the sphere of chromatic substance after growing may break up into several parts, or it may remain discrete. In Apanteles the larger secondary nuclei resemble the true or head nucleus to the smallest details, and one who did not know that these bodies had originated in a peculiar manner would unhesitatingly pronounce them to be normal nuclei, capable of undergoing mitosis (Pl. 9, fig. 11, *S.N.*).

Some time before the ovarian oöcyte has become ripe the secondary nuclei disappear by a process of degeneration or chromatolysis. The nuclear wall disintegrates and the other secondary nuclear elements become indistinguishable from the ground cytoplasm.

5. The Mitochondria.

All three ovarian elements, egg, nurse- and follicle-cell, have a common derivation as explained. The indifferent cell is drawn in Pl. 9, fig. 1; the mitochondria at *M.* are fairly conspicuous granular formations. In each ovarian element the mitochondria have a special character; in the follicle-cells they remain unchanged (Pl. 9, figs. 6, 11); in the nurse-cells they change profoundly, becoming extremely numerous and fine. They are then closely grouped around the nucleus. In Pl. 9, fig. 7, is a young nurse-cell; its mitochondria are not much finer than those of the indifferent cell, but are more numerous. Gradually these mitochondria divide and multiply till they form a dust-like halo around the nucleus (Pl. 9, figs. 8 and 9, *M.*).

Inferior fixation causes the mitochondria to collapse and fuse partly to form a number of concentric rings around the nurse-cell nucleus; even in the best preparations the very fine mitochondria form a thick impenetrable layer around the nurse-cell nucleus. The rest of the nurse-cell cytoplasm has some mitochondria in it, but less closely packed.

In the follicle-cells the mitochondria keep large, and much like those of the indifferent cell (see Pl. 9, figs. 4, 5, and 11).

The history of the mitochondria in the egg is quite different. In the young oöcyte undergoing the early prophases of the maturation divisions as in Pl. 9, figs. 2 and 3, the mitochondria are coarsely granular and tend to lie around an archoplasmic sphere (*A.R.*). The Golgi apparatus will be found to lie upon this sphere, but so far I have been unable to demonstrate it either by Kopsch or Cajal technique. In the larger insect Dytiscus, a water-beetle, Nusbaum-Hilarowicz has shown a Golgi apparatus to be present in ovarian and follicle cells. It takes the form of small rings or half hoops very like that of Helix (5) or Lepidoptera (6). The granular oöcyte mitochondria gradually spread around the nuclear periphery as in Pl. 9, fig. 4. About this time they lose their granular appearance and become at first elongate and then filiform.

In Pl. 9, fig. 5, this is just happening. After this stage the granules tend to pass outwards through the cytoplasm, often leaving a space around the nucleus, *CS.* in Pl. 9, fig. 6. Notice in this figure the difference between follicular (*F.*) and oöcyte (*M.*) mitochondria. Just after the stage in Pl. 9, fig. 6, the mitochondria pass throughout the entire egg cytoplasm (fig. 10), and yolk begins to appear at the periphery of the oöcyte. In later stages the mitochondria increase enormously in importance and may form thick matted masses in the egg (Pl. 9, fig. 11, *M.*).

6. The Yolk (Deutoplasmogenesis).

After the mitochondria have spread throughout the egg and have become filamentary, there ensues a period in which the periphery of the egg becomes markedly well supplied with a matted dense cloud of mitochondria (Pl. 9, fig. 6).

As yet no yolk has appeared; the first sign of yolk is found in the appearance of tiny spheres close to the periphery of the egg, beneath the follicle wall and in the mat of mitochondria. As the egg grows older (Pl. 9, fig. 11, *Y.D.*) the yolk-spheres become much larger, but still are found only around the peripheral layer on the oöcyte.

Subsequently the spheres spread inwards, becoming larger as they do so, and eventually the entire substance of the egg is found to be loaded with the spheres. It is during this process that the secondary nuclei attain their largest size; as has been remarked, the latter lie on the periphery of the egg as shown in Pl. 9, figs. 11 and 12, and in Text-fig. 1, and the inference is that they are placed there in order that their influence may be used in causing the formation of food-yolk.

In no case did I find anything which would suggest that yolk was formed by metamorphosis of the egg mitochondria, and I consider that this is not a usual occurrence in oögenesis.

7. The Composition of the Germ-Cell Determinant.

The following fixing and staining tests were carried out on the germ-cell determinant.

Chrome-osmium.—Fixation of the ovary in Flemming-without-acetic and staining in iron-hæmatoxylin and orange G or in safranin gave the result drawn in Pl. 9, fig. 12. The determinant before staining looked a yellowish-brown colour, and after staining a dark brown to black colour. It was made up of granules, in the early stages at least (such as in fig. 12), and these grains were spherical and seemed something apart from the rest of the egg cytoplasm. No cytoplasmic reticulum to be seen in preparations fixed as above. The mitochondria appeared in the egg as fine filaments, generally several tangled and matted as in Pl. 9, fig. 11, *M.*

Mitochondria were found quite close to and often seemingly at the edge of the substance of the germ-cell determinant. Yolk-discs were yellowish by this method and beautifully preserved; they did not actually lie in the substance of the germ-cell determinant, but some passed around under the posterior end of the determinant as at *Y.D.X.* in Pl. 9, fig. 12.

Kopsch's Method.—This method consists in leaving material in 2 per cent. OsO_4 for two weeks. By it fat and generally Golgi apparatus are blackened; lecithin and ground protoplasm go a yellowish to light brown in Kopsch. In Pl. 9, fig. 13, I have drawn the posterior pole of an egg at the same stage as in Pl. 9, fig. 12.

This test shows that the area of activity of the formation of the germ-cell determinant is not confined to the inner region of the pole of the egg, as in Pl. 9, fig. 12, but is spread over the entire posterior pole; this is indicated by the fact that while the darkest part of the egg lies at *G.C.D.*, in Pl. 9, fig. 13, the area immediately below and at the sides is much darker than the cytoplasm not in the region of the germ-cell determinant. No structure or area in the egg at this stage could be made to go black with OsO_4, the natural inference consequently being that the germ-cell determinant does not contain fat (olein).

Fixation of the ovaries in picro-nitric has the effect of removing all the yolk and apparently all signs of mitochondria.

Even though this treatment removes all yolk-discs as shown in Pl. 9, fig. 14, it has not the effect of removing all the germ-cell determinant or of making it less obvious after staining. The natural inference from this is that while the germ-cell determinant may possibly contain some free lecithin, its main bulk consists of some organic substance other than lecithin or fat.

Fixation of the oöcytes in Carnoy or absolute alcohol and staining for glycogen by the iodine method and by Best's carmine did not succeed in tinging the region of the determinant in any way suggesting that it contained glycogen, while the fact that the determinant is well preserved by fixatives known to dissolve away glycogen leaves us with the conclusion that the determinant consists of something other than glycogen.

Methods such as that of Kopsch and Cajal for the Golgi apparatus failed to reveal this latter either in egg or in germ-cell determinant. That this shows that the oöcyte of Apanteles does not contain a Golgi apparatus is a conclusion which I am unwilling to draw, but from other tests, such as that of Carnoy's fluid (known to dissolve the Golgi apparatus of other eggs), I feel justified in concluding that the Golgi apparatus does not form a part of the germ-cell determinant (see also pp. 140 and 149).

Especially after fixation of the ovary in alcohol and acetic fixatives, the germ-cell determinant stains basophil, but this alone does not show that it contains chromatin. We have no evidence that the germ-cell determinant is even partly formed of chromatin, while we cannot observe any occurrences in the history of the formation of the determinant which lead us to associate its inception and growth with either head nucleus or secondary nuclei. It should be mentioned that it is not possible to obtain completely satisfactory evidence that the germ-cell determinant does not contain chromatin, but I feel sure that chromatin in bulk masses does not take part in forming the determinant. Granulose emissions of chromatin might pass into the determinant from the nucleus without the

fact being observable. Since, however, the determinant does not stain quite like chromatin one can only believe that the evidence is against the assumption that this protoplasmic aggregation is formed partly or wholly of chromatinic substance.

8. Discussion.

(a) Germ-cell Determinant.

In this paper it has been shown that the germ-cell determinant in Apanteles glomeratus is probably formed of albuminous material as opposed to chromatin, or storage substances such as fat, yolk, or glycogen. The exact significance of this is difficult to determine, but it seems probable that germ-cell determinant is a form of metaplasm, and that its purpose is that of providing nutriment in a special form, enabling the germ-cells to remain independent of the other cells, such as those of the germ-layers, while the latter are undergoing their organogeny changes. Histo-chemically the germ-cell determinant consists of proteid, which, when coagulated by alcohol, acetic acid, corrosive sublimate, etc., becomes insoluble in either water, alcohol, or xylol and chloroform.

No direct connection can be shown to exist between the germ-cell determinant and the chromatin of the oöcyte nucleus or the follicular nuclei. It must be admitted, however, that this does not show positively that the germ-cell determinant contains no chromatin. The germ-cell determinant, while being basophil, never stains in quite the same sharp way as the chromatin of the nucleus when one uses dyes like methyl green or safranin; for this reason alone one is justified in considering that the germ-cell determinant is at least not formed of nucleo-proteid of the same constitution as that found in the nucleus.

(b) The Secondary Nuclei.

The secondary or accessory nuclei of the insects are very remarkable structures, and there is no doubt that they throw a great deal of light on the general structure of a

nucleus. Among the several interesting facts which have been ascertained with regard to these accessory nuclei, none is more remarkable than that which shows them to be of nuclear origin and to contain chromatin. The small granules found in the cytoplasm which give rise to the accessory nuclei stain exactly like chromatin, and personally I have no doubt that such granules are chromatin. These granules lie naked in the cytoplasm, but later form around themselves a nuclear membrane and a linin network. The enchylema or nuclear sap just after formation is quite hyaline, and the linin appears later. Buchner has pointed out that the study of the accessory nuclei shows that the presence of chromosomes is not a *sine quâ non* for the building up of a nucleus. This statement, however, is not quite true, nor is it a new suggestion. What is a chromosome? It is merely a mass of chromatin, which appears in the nucleus during karyokinesis, and I believe that it is not possible to discriminate between a large piece of extruded chromatin (Text-fig. 9) and a chromosome in the sense meant by Buchner. We also were aware previous to Buchner's work that single chromosomes could form nuclei (**2**, **6**).

By far the most important result of work on the accessory nuclei is the fact that chromatin, apparently in large quantities, can be produced in and budded off from a germinal vesicle without affecting the subsequent results of development. We have distinctly satisfactory evidence that the chromatin forming the accessory nuclei passes out of the germinal vesicle, but none that any passes back, while there is good evidence that the accessory nuclei degenerate eventually.

The egg nucleus in many insects, of which Apanteles is an example, becomes partly decentralised; this is to say, the nucleus, instead of influencing various processes of oögenesis from afar, sends pieces of itself into the furthermost regions of the egg, which carry on part of the vegetative functions at least of the chromatin in the ordinary nucleus. The head nucleus is able to produce chromatin for this purpose, without endangering the complete exercise of its proper functions in

later stages of the germ-cell cycle. The chromatin so produced must apparently be part of the chromosomes of the egg nucleus, so that one seems led to believe that the chromosomes are able to part with some of their substance without detriment. This phenomenon, as also the appearance of giant germ-nurse cells in Testacella (5), is in some way connected with the growth faculty of the substance "chromatin." In the case of Apanteles and of Testacella, the new chromatin so produced might by some be looked upon as trophochromatin, or specialised for purely trophic or vegetative functions.

In certain cases, such as that of Apanteles, the secondary nucleus exactly resembles the head nucleus except for size. This applies also in such an example as Rhyssa, in Text-fig. 7. In other cases, however, the secondary nuclei may not resemble the head nucleus, and may be much larger than the latter, as in Text-fig. 6, of Camponotus. This difference in appearance seems due to the difference in the arrangement and denseness of the linin network in the accessory nuclei; in the latter the karyolymph is more dense, or the linin is more closely woven.

It seems indicated that the secondary nuclei are formed from true chromatin of the head nucleus, and that their function is connected with the growth of yolk or other materials in the egg-cytoplasm. All staining tests appear to show that such chromatin extruded from the nucleus is exactly similar in its histo-chemistry to the chromosomes. It should be mentioned that Dendy (3a) especially shows that in several sponges solid lumps of chromatin are extruded from the nucleus of the egg during oögenesis, this also without affecting subsequent development of the organism.[1]

I mention these facts, not as a disbeliever in the useful "chromosome hypothesis," but because I consider that chromosome theorists might be interested enough to comment on such peculiar occurrences, which seem fairly wide-spread, and which must be explained by workers on chromosomes.

[1] I have seen these preparations recently.

(c) The Protoplasmic and Deutoplasmic Inclusions of Insect Germ-Cells.

In the Lepidoptera it has been shown that the male germ-cells contain both mitochondria and Golgi apparatus (6); I consider that the sickle-shaped bodies drawn by me in the figures of the sperm-cells of moths are homologues of the Golgi apparatus or "apparato interno reticolare" of the mammalian nerve or germ-cell. In my work on moths I failed to demonstrate such sickle-shaped bodies in the öocyte; likewise in this present paper all my efforts to show such bodies in the oögenesis of Apanteles have failed.[1] It is therefore interesting that a Polish worker, Nusbaum-Hilarowicz, should have succeeded in demonstrating a Golgi apparatus in the young oöcytes of another insect—the large water-beetle, Dytiscus. This has been done by using a special new modification of Kopsch's method. In examining male germ-cells of Apanteles I have found the Golgi apparatus, and I believe that some special modification of Kopsch's method should also show the apparatus in the egg, as has been done by me in Limnæa and other molluscs. This matter, however, does not affect the issues discussed in this present paper.

In Apanteles the oöcyte contains no fat-droplets such as have been shown to occur in the egg of Dytiscus, but in both insects yolk-spheres are present and appear in the same region of the egg cytoplasm. Nusbaum-Hilarowicz shows that yolk-spherules and fat-droplets differ in their histo-chemical reactions, as has already been pointed out elsewhere (7).

9. Conclusions.

Composition.

(1) The germ-cell determinant of Apanteles glomeratus is formed almost wholly of a basophil albuminous proteid somewhat like that of the ground protoplasm of the egg-cell, only much more dense and definitely basophil.

[1] I have made preparations of Stenobothrus ovary showing Golgi elements, by Cajal's method and by Mann-Hopsch. Apanteles is small and difficult to manipulate by Golgi techniques.

(2) This substance forming the germ-cell determinant of Apanteles is partly gathered into the form of fine granules. Fat solvents and lipin solvents tend to destroy the granular formation, but accentuate and make more chromophil the ground substance of the germ-cell determinant. After fat solvents the latter becomes stringy.

(3) Solvents which disintegrate the yolk-spheres (fat and lecithin) do not remove the germ-cell determinant from the egg.

(4) Tests for fat do not reveal the presence of this substance in the germ-cell determinant.

(5) Fixations which remove the mitochondria from the egg do not remove the germ-cell determinant, while after chrome-osmium fixation, staining methods which do not reveal the mitochondria stain the region of the germ-cell determinant in a basophil manner.

(6) Glycogen methods did not reveal any substance in the determinant which could be identified as glycogen.

(7) The test made for demonstrating the Golgi apparatus (Kopsch's method and that of Cajal) did not bring into evidence any structure which might have been identified as such.

Origin.

(8) The germ-cell determinant originates as a concentrated area at the posterior pole of the young oöcyte. At first it is merely a region of the egg cytoplasm denser than the surrounding, but later it becomes more clearly marked off. No evidence was found supporting the suggestion that the germ-cell determinant contains chromatin. At the time of origin of the determinant the oöcyte nucleus lies at the opposite end of the egg. The secondary or accessory nuclei have no connection with the germ-cell determinant.

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EXPLANATION OF PLATE 9,

Illustrating Mr. J. Bronté Gatenby's paper "On the Germ-Cell Determinant of Parasitic Hymenoptera."

Lettering.

AR. Archoplasm with mitochondria (?). *CS.* Space in oöcyte free from mitochondria. *F.* Follicle. *FW.* Follicle wall. *FOM.* Outer follicle membrane. *GCD.* Germ-cell determinant. *M.* Mitochondria. *MN.* Nurse-cell mitochondria. *N.* Nucleus. *NCN.* Nurse-cell nucleus. *N.NCN.* Nucleolus of nurse-cell nucleus. *OVW.* Ovariole wall. *PR.OCYTE.* Protoplasm of oöcyte. *SN.* Secondary nuclei. *VA.* Vacuoles (?). *X.* Bodies of uncertain nature, possibly fat. *YD.* Yolk-discs or granules.

In the middle of the plate scales for the figures are given.

Fixation indicated by the letters: *F.W.A.* Flemming-without-acetic acid. *CH.* Champy. *B.* Bouin's picro-formol acetic. *P.N.* Picro-nitric. *K.* Kopsch.

[All figures drawn from material stained in iron-hæmatoxylin, with the exception of fig. 13. Camera lucida, paper at table level, $\frac{1}{15}$ semi-apochromatic Koritska oil-immersion and compensating eyepieces.]

PLATE 9.

(All figures of Apanteles glomeratus.)

Fig. 1.—Transverse section of upper part of ovariole in region where the cells are as yet undifferentiated into oöcytes and nurse- and follicle-cells. Each cell has a completely differentiated mitochondrial apparatus (*M.*). The ovariole wall is at *OVW.* $\frac{1}{4}$ dilute *F.W.A.*

Fig. 2.—Section of ovariole further down cut longitudinally in region of differentiation of nurse- and follicle cells. The inner cell (*PR.OCYTE*) will be the egg; the other cells will form either follicle- or nurse-cells. In the oöcyte the mitochondria have become grouped around an archoplasm. $\frac{1}{4}$ dilute *F.W.A.*

Fig. 3.—Somewhat older oöcyte showing same cytoplasmic contents, and in addition three or four bodies (X^1, X^3) of doubtful nature, possibly oil or fat. *Ch.*